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**UTILITY PATENT APPLICATION TRANSMITTAL**  
**(Large Entity)***(Only for new nonprovisional applications under 37 CFR 1.53(b))*Docket No.  
1030-R-00Total Pages in this Submission  
171**TO THE ASSISTANT COMMISSIONER FOR PATENTS**Box Patent Application  
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

A Method For The Identification of Anesthetics

and invented by:

Amanda J. PATEL et al.

jc135 U.S. PTO  
09/503089  
02/11/00If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:☐ Continuation ☐ Divisional ☒ Continuation-in-part (CIP) of prior application No.: 09/144,914

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Enclosed are:

**Application Elements**

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 21 pages and including the following:
  - a. ☒ Descriptive Title of the Invention
  - b. ☒ Cross References to Related Applications *(if applicable)*
  - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
  - d. ☐ Reference to Microfiche Appendix *(if applicable)*
  - e. ☒ Background of the Invention
  - f. ☒ Brief Summary of the Invention
  - g. ☒ Brief Description of the Drawings *(if drawings filed)*
  - h. ☒ Detailed Description
  - i. ☒ Claim(s) as Classified Below
  - j. ☒ Abstract of the Disclosure

# UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.  
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## Application Elements (Continued)

3. ☒ Drawing(s) (when necessary as prescribed by 35 USC 113)
- a. ☐ Formal Number of Sheets \_\_\_\_\_
- b. ☒ Informal Number of Sheets 5
4. ☒ Oath or Declaration
- a. ☐ Newly executed (original or copy) ☒ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)
- c. ☒ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named in the prior application,  
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference (usable if Box 4b is checked)  
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied  
under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby  
incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all must be included)
- a. ☒ Paper Copy
- b. ☒ Computer Readable Copy (identical to computer copy)
- c. ☒ Statement Verifying Identical Paper and Computer Readable Copy

## Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(B) Statement (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☒ Information Disclosure Statement/PTO-1449 ☒ Copies of IDS Citations
12. ☐ Preliminary Amendment
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# UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

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## Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

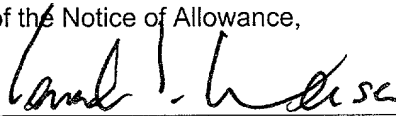
16. ☐ Additional Enclosures (please identify below):

## Fee Calculation and Transmittal

### CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	25	- 20 =	5	x \$18.00	\$90.00
Indep. Claims	8	- 3 =	5	x \$78.00	\$390.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$690.00
OTHER FEE (specify purpose)					\$0.00
TOTAL FILING FEE					\$1,170.00

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Dated: February 11, 2000

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**CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)**Applicant(s): **Amanda J. PATEL et al.**

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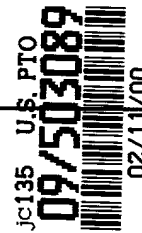
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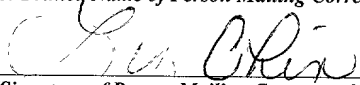
Group Art Unit

Not yet assigned

Invention: **A Method For The Identification of Anesthetics**I hereby certify that this **Utility Patent Application and accompanying documents***(Identify type of correspondence)*

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under  
37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231

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**APPLICATION FOR UNITED STATES LETTERS PATENT**

**FOR**

**A METHOD FOR THE IDENTIFICATION OF ANESTHETICS**

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## BACKGROUND OF THE INVENTION

This patent application is a continuation-in-part of pending application Serial No. 09/144,914 filed September 1, 1998, which is incorporated by reference in its entirety. This patent application also claims the priority of U.S. Provisional Application No. 60/119,727, filed February 12, 1999.

### 1. Field of the Invention

The invention relates to a method for identifying substances that are capable of acting as anesthetics.

### 2. Background of the Related Art

Volatile anesthetics are a remarkable class of agents producing a safe, reversible state of unconsciousness with concurrent amnesia and analgesia. They have hyperpolarizing action on mammalian neurons. They activate an inhibitory synaptic  $K^+$  current ( $I_{K(An)}$ ) in molluscan pacemaker neurons which has been proposed to have an important role in general anaesthesia.

Volatile anesthetics hyperpolarize frog motor neurons, rat hippocampal neurons, guinea pig thalamic neurons and human cerebral cortex neurons. Therefore, it has been proposed that the molecular mechanism of volatile anesthetics involves an action on a specific class of  $K^+$  channels. The fact that a particular inhibitory synaptic  $K^+$  current,  $I_{K(An)}$ , reversibly activated by volatile agents is present in anesthetic-sensitive molluscan pacemaker neurons, but absent in insensitive neurons has made it a very attractive candidate as a target for these important pharmacological agents.  $I_{K(An)}$  behaves as a background channel; it is not voltage-gated, it activates immediately, and it does not inactivate with time.  $I_{K(An)}$  obeys the Goldman-Hodgkin-Katz constant field equation and is resistant to the classical  $K^+$  channel blockers, tetraethylammonium and 4-aminopyridine.

We recently identified a novel family of mammalian  $K^+$  channels with a unique structural

motif consisting of two pore domains in tandem and four transmembrane segments. The four members in this family have been classified as TWIK-1, TASK, TREK-1, and TRAAK, which are shown in Fig. 1A. TWIK-1 has been previously shown to dimerize, implying that a functional channel is formed by at least two subunits. Heteromultimerization does not occur between the four members of this novel family as tested in Sf9 cells expressing various combinations of these channels (unpublished data). Three members of this family, mouse TREK-1, mouse TRAAK, and human TASK, encode for background outward-going K<sup>+</sup> rectifiers with properties resembling those of I<sub>K(An)</sub>. TRAAK and TREK-1 are directly opened by arachidonic acid and other polyunsaturated fatty acids, while TASK encodes a resting K<sup>+</sup> channel which is controlled by external pH variations near physiological pH. TREK-1 and TASK are expressed in many tissues and are particularly abundant in the brain and in the heart, whereas TRAAK is selectively expressed in the central nervous system. Neuronal expression of these channels is detected at high levels in the cortex, cerebellum, hippocampus and olfactory bulb, and cardiac expression is detected in both the myocardium and connective tissues.

## BRIEF DESCRIPTION OF THE FIGURES

**Fig. 1** shows the results of experiments with chloroform demonstrating that chloroform selectively activates TREK-1: A, a schematic showing the two pore domains and four transmembrane domains of the 2P domain potassium K<sup>+</sup> channels, TREK-1 TRAAK, TWIK-1 and TASK. The four transmembrane domain segments are indicated by TM1 through TM4, and the two pore regions are indicated by P1 and P2. The phylogenetic tree indicates the three subfamilies. TWIK-1 is an inward rectifier K<sup>+</sup> channel and TASK is a background rectifier K<sup>+</sup> channel inhibited

by external acidosis. Both TREK-1 and TRAAK are background outward rectifier K<sup>+</sup> channels opened by arachidonic acid; B, whole cell patch clamp experiments showing that 0.8 mM chloroform strongly and reversibly activates TREK-1 expressed in transfected COS cells. The mock condition is the wild-type (empty) expression vector. The effects of chloroform were investigated on K<sup>+</sup> currents elicited in the whole cell configuration during a voltage ramp of one second in duration from a holding potential of -80 mV as illustrated in the inset for TREK-1; C, 0.8 mM chloroform induced a typical background current characterized by an outward-going rectification which reverses at the predicted value for E<sub>K<sup>+</sup></sub>; Chloroform activated TREK-1 currents in physiological and symmetrical K<sup>+</sup> gradients were examined. Voltage ramps of one second in duration from a holding potential of -80 mV in both K<sup>+</sup> conditions and in the presence of 0.8 mM chloroform were digitally subtracted from ramps in control conditions; D, 0.8 mM chloroform reversibly and reproducibly hyperpolarized COS cells expressing TREK-1; E, dose-dependence of TREK-1 activation. The inset illustrates the effect of 0.8 mM chloroform on TREK-1 current measured at a holding potential of 0 mV. The number of cells in each experimental condition is indicated.

**Fig. 2** Halothane is a common activator of TREK-1 and TASK. A, comparative effects of 1 mM halothane on the 2P domain K<sup>+</sup> channel activities. The mock condition is the wild-type (empty) expression vector; B, halothane (1 mM) stimulates TASK channel activity elicited in the whole cell configuration during voltage ramps of one second in duration from a holding potential of -80 mV; C, halothane (1 mM)-activated TASK currents in physiological and symmetrical K<sup>+</sup> gradients. Voltage ramps of one second in duration from a holding potential of -80 mV in both K<sup>+</sup> conditions and in the presence of 1 mM halothane are digitally subtracted from ramps in control

conditions; D, dose-effect curve of halothane on TREK-1 channel activation; F, halothane (1 mM) reversibly activates TASK at a holding potential of 0 mV. The number of cells in each experimental condition is indicated.

**Fig. 3** Isoflurane and diethyl ether differentially activate TREK-1 and TASK. A, comparative effects of 2 mM isoflurane (A) and 0.6 mM diethyl ether (B) on the 2P domain K<sup>+</sup> channels. The mock condition is the wild-type (empty) expression vector. The effects of the anesthetics are investigated on K<sup>+</sup> currents elicited in whole cell configuration during voltage ramps of one second in duration from a holding potential of -80 mV as illustrated in insets for TREK-1. The number of cells in each experimental condition is indicated.

**Fig. 4** Volatile anesthetics stimulate TREK-1 and TASK in the excised patch configurations. A, effects of increasing concentrations of halothane on TREK-1 channel activity in an outside-out patch. In an outside-out patch, halothane reversibly opens a 48 pS TREK-1 channel in a dose-dependent fashion. The holding potential is 0 mV and applications of halothane are indicated by horizontal bars; B, effect of 0.8 mM chloroform on the I-V curve of TREK-1 in an outside-out patch. The I-V curve is performed with a voltage ramp of one second in duration from a holding potential of -80 mV; C, kinetics of activation of TREK-1 by 0.8 mM chloroform. The I-V curve of the chloroform-sensitive current in an outside-out patch shows the characteristic outward-going rectification; D, halothane (1 mM) induces TASK channel opening in an inside-out patch. The holding potential is 0 mV and channel activities before, during, and after addition of 1 mM halothane are illustrated from left to right. Halothane reversibly opens a 12 pS TASK channel.

**Fig. 5** Functional characterization of the human TREK-1 (hTREK-1). Transiently transfected COS cells expressing hTREK-1 are voltage-clamped using the whole cell patch clamp

configuration. A, The basal TREK-1 current is recorded in physiological K conditions (5 mM) and in symmetrical K conditions (155 mM). The cells are held at -80 mV and voltage ramps of 800 ms in duration are applied from -130 mV to 100 mV. B, hTREK-1 is stimulated by the addition of 10  $\mu$ M arachidonic acid in the bath. Same protocol as in A. C, hTREK-1 is opened by increasing concentrations of halothane (as indicated). D, In the inside-out patch configuration hTREK-1 is opened by a membrane stretch of -66 mmHg. The patch is voltage clamped at 0 mV.

### SUMMARY OF THE INVENTION

The invention relates to human TREK-1, its nucleotide and amino acid sequence. The invention further relates to murine TREK-1, its nucleotide and amino acid sequence.

Thus, the invention relates to a method for the identification of substances that activate potassium transport through a potassium transport protein, including TREK-1 and TASK. The potassium transport proteins activated in the method exhibit outward-going rectification. Specifically, the method involves contacting a substance to be tested with a mammalian transport protein and examining the potassium transport activity of the potassium transport proteins. A positive result, activation of potassium transport, correlates with a substance which produces anesthesia.

### DETAILED DESCRIPTION OF THE INVENTION

The invention is an isolated nucleic acid molecule encoding the human TREK-1 channel (SEQ ID NO:1). The invention is also embodied in the isolated human TREK-1 protein (SEQ ID NO:2). The nucleic acid and deduced amino acid sequence is shown in SEQ ID NO:1.

The invention also encompasses the isolated nucleic acid molecule encoding the murine TREK-1 channel (SEQ ID NO:3). The isolated protein, murine TREK-1 (SEQ ID NO:4), is also

encompassed by the invention.

The method involves contacting a test substance with a potassium transport protein *in vitro* and determining whether there has been activation of potassium transport.

As used in the method, cells which express a potassium transport protein are used in the presence of the test substance. The test substance is a substance which will have certain properties when used in a mammal as a volatile inhalant. These properties may include the induction of a safe, reversible state of unconsciousness, amnesia and analgesia.

The cells expressing the potassium transport protein may transiently express the protein or constitutively express the protein. The cells may be of any type which can express the protein in appropriate conformation to allow for the transport of potassium. Examples of such cells include, mammalian cells, vertebrate cells, and invertebrate cells. Examples of mammalian cells suitable for use in the invention include, but are not limited to, cells of neuronal origin, fibroblasts, myocardial cells, COS cells, Chinese hamster ovary (CHO) cells, embryonic kidney cells, fibroblasts, HELA cells, and the like. Examples of suitable non-mammalian vertebrate cells include, but are not limited to frog oocytes, such as *Xenopus laevis* oocytes, and the like. Suitable invertebrate cells include, but are not limited to insect cells, such as *Spodoptera frugiperda* (Sf9) cells, and the like. Any cell known in the art which may be transfected to transiently or constitutively express the transport protein are suitable for use in the present invention.

The potassium transport proteins which are suitable for use in the invention include TREK-1 (SEQ ID NO:2 and SEQ ID NO:4) and TASK (SEQ ID NO:5), which may be derived from any mammalian source, such as rat, mouse, or human. The molecular sequence of TREK-1 may be the human TREK-1 as shown in SEQ ID NO:2, or an amino acid sequence that is substantially identical



voltage clamp with a Dagan TEV200 amplifier, The bath solution contains 98 mM KCl, 1.8 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, and 5 mM HEPES at pH 7.4 with KOH.

Alternatively, functional expression of the potassium channel may be accomplished by transfection of insect cells, such as *Spodoptera frugiperda* (Sf9) cells. Briefly a suitable vector, such as pVL1392 may be used and the coding sequence for the potassium transport protein may be inserted into the vector in-frame so that expression of the potassium transport protein may be expressed. The coding sequence for the potassium transport protein may be obtained by any convenient method, such as by PCR or by digesting a plasmid containing the potassium transport protein coding sequence with appropriate restriction endonuclease(s) for subsequent ligation into the pVL1392 vector. Similarly, the amplified product of the PCR may be digested with restriction enzymes and ligated into the vector. Transfection of SF9 cells may be performed by the manufacturer's protocol (Pharmingen).

Alternatively, functional expression of the potassium channel may be by transient transfection of cells such as COS cells whereby COS cells are seeded at a density of 20,000 cells per 35 mm dish. Cells are transfected with expression vector, such as the pIRES-CD8 vector, comprising the nucleic acid molecules encoding the desired potassium channel protein. The cells may be transfected by any method known in the art, such as the DEAE dextran protocol, Ca<sub>2</sub>PO<sub>4</sub> precipitation, or electroporation, for example. The transfected cells, expressing the desired potassium channel, or induced to express the desired potassium channel may then be used in the method of the invention and the cells may be assayed for the transport of potassium.

The invention will be described in greater detail with reference to the examples which are provided to illustrate the invention. The examples are not to be construed to be limiting as to the

scope of the invention, which is set forth in the appended claims.

## EXAMPLES

### 1. Cloning of the human TREK-1 channel

The TREK-1 channel was cloned by degenerate PCR technology using previously characterized murine sequence. Although such technology is extensively described in the literature and is familiar to those of ordinary skill in the art, briefly, degenerate oligonucleotide primers selected to amplify a region of murine TREK-1 were synthesized and placed in a polymerase chain reaction amplification using human DNA with appropriate buffer, nucleotides and DNA polymerase. The reaction is cycled through temperature stages for denaturation of DNA (generally about 94°C), annealing of primers to DNA template (this temperature can be varied to optimize amplification and can be based on many factors, including primer length and GC content), and extension of DNA polymerization by DNA polymerase (generally at the optimum temperature for the activity of the DNA polymerase which is usually about 72°C). The amplified DNA fragment may be isolated and cloned into a plasmid vector for subsequent sequence analysis, or the amplified DNA may be directly sequenced by known methods.

Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the human TREK-1 channel that encodes the amino acid sequence determined for murine TREK-1 (SEQ ID NO:2). Moreover, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein. Such substitutions are known in the art as "conservative substitutions." The invention

encompasses human TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered. Generally, the identity of such an mutant TREK-1 will be at least 90% identical to SEQ ID NO:2. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:2. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:2. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:2.

## **2. The sequencing of the murine TREK-1 channel**

The sequence for murine TREK-1, which is a corrected form of murine TREK-1 reported earlier, is shown in SEQ ID NO:4. There is a longer open reading frame than originally reported, producing a protein with a deduced amino acid sequence of 411 amino acids. Due to the degeneracy of the DNA code, it will be well understood to one of ordinary skill in the art that substitution of nucleotides may be made without changing the amino acid sequence of the protein. Therefore, the invention includes any nucleic acid sequence for the murine TREK-1 channel that encodes the amino acid sequence determined for murine TREK-1 (SEQ ID NO:4). Moreover, as is the case with human TREK-1, it is understood in the art that for a given protein's amino acid sequence, substitution of certain amino acids in the sequence can be made without significant effect on the function of the protein. The invention encompasses murine TREK-1 proteins that contain conservative substitutions, wherein the function of the protein is not altered. Generally, the identity of such an mutant TREK-1 will be at least 90% identical to SEQ ID NO:4. Preferably, the mutant TREK-1 will be at least 95% identical to SEQ ID NO:4. More preferably, the mutant TREK-1 will be at least 97% identical to SEQ ID NO:4. Most preferably, the mutant TREK-1 will be at least 99% identical to SEQ ID NO:4.

## **3. Functional expression of the human TREK-1 channel**

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The functional properties of the human TREK-1 channel were studied on the basis of transfected COS cells temporarily expressing the protein. Like the murine channel, the human channel is selective for potassium and is activated by *cis*-unsaturated fatty acids, volatile anesthetics and by stretching of the plasma membrane. The selectivity of the TREK-1 currents for potassium is shown in Fig. 5A. The currents are recordings in the whole-cell configuration of the patch-clamp technique (imposed potential gradient from -130 to +100 mV). The inverse potential of the currents follow the equilibrium potential of potassium when the extracellular concentration of potassium ions climbs from 5 to 155 mM. Fig. 5B shows that the application of arachidonic acid (10  $\mu$ M) induced activation of the TREK-1 currents. Fig. 5C shows that the application of volatile anesthetics (halothane in this case) at the concentrations employed in general anesthesia induced activation of the TREK-1 channel. Fig. 5D shows that the TREK-1 currents recorded in an excised patch (inside-out configuration) are mechanosensitive. When a pressure of -66 mmHg is applied on the membrane, the TREK-1 currents are activated in a reversible manner.

#### 4. Electrophysiological recording

COS cell transfection, culture and electrophysiology are well known in the art and are described in the literature such as in the references cited.

As performed herein, COS cells were seeded at a density of 20,000 cells per 35 mm dish 24 hours prior to transfection. Cells were transfected by the DEAE dextran protocol (1  $\mu$ g DNA per plate). Mouse TREK-1 (GenBank Accession No. U73488), human TASK (GenBank Accession No. AF006823) and mouse TRAAK (GenBank Accession No. AF056492) fragments were amplified by polymerase chain reaction (PCR) and subcloned into the pIRES-CD8 vector. Transfected cells were visualized 48 hours after transfection using the anti-CD8 antibody coated beads method. For whole

cell and excised patch experiments, the internal solution was 150 mM KCl, 3 mM MgCl<sub>2</sub>, 5mM EGTA and 10 mM HEPES at pH 7.2 with KOH and the external medium contained 150 mM NaCl, 5 mM KCL, 3 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 10 mM HEPES at pH 7.4 with NaOH. Cells were continuously superfused with a microperfusion system during the time course of the experiments (0.1 ml/min) performed at room temperature. A RK300 patch clamp amplifier (Biologic, Grenoble, France) was used for whole cell as well as single channel recordings. Ionic currents were monitored and recorded continuously using a DAT recorder (Biologic, Grenoble, France). Subsequently, data were replayed and sampled using pClamp software. Data analysis were performed using clampfit (pClamp) for whole cell recording as well as Biopatch (Biologic) software for single channel recordings. Membrane capacitance was measured during a 10 V hyperpolarizing step from a holding potential of -80 mV. Student's t test was used for statistical analysis (P<0.001).

## 5. Anesthetics delivery and concentration

General anesthetics were directly dissolved in saline solutions. All experiments were performed at room temperature (24°C). Solutions were prepared every 3 hours in gas-tight bottles as stock solutions (calculated concentrations of 5 10 mM). Serial dilutions were prepared just prior to the electrophysiological experiment. 2.5 ml of each experimental solution at the desired concentration was placed in a syringe connected to the experimental superfusion system. The electrophysiological measurements were performed within 45 minutes.

The actual concentrations of anesthetics were subsequently determined by means of a gas chromatography method (HP 6890 equipped with a DB624 column) using FID detection. Sampled (2.5 ml of solution) were collected prior to (t<sub>0</sub>) and after perfusion (t<sub>45</sub>) through the experimental setup. Solutions were collected using gas impermeable tubing and stored in sealed glass containers

at 4°C for subsequent analysis. Samplings and measurements were performed in duplicate. Actual concentrations of anesthetics were determined by multiplying the calculated concentration by the ratio  $t_{45}/t_0$  (chloroform: 0.16; halothane: 0.37; isoflurane: 0.76; and diethyl ether: 0.57). In the dose effect curves, the threshold concentrations were estimated as concentrations producing an increase higher than 10% in current amplitude.

## RESULTS

The nucleic acid sequence corresponding to the open reading frame of the human TREK-1 channel is shown in SEQ ID NO:1. The sequence of 1236 nucleotides encodes a protein of 411 amino acids. The conservation between human and murine proteins is very high, exceeding 99% homology.

TASK and TREK-1, two mammalian 2P domain K<sup>+</sup> channels which have similar properties to  $I_{K(An)}$  are activated by volatile general anesthetics. Chloroform, diethyl ether, halothane and isoflurane activated TREK-1, while only halothane and isoflurane activated TASK. The C-terminal regions of both TREK-1 and TASK are critical for anesthetic activation. Thus, both TREK-1 and TASK are important target sites for these agents.

In whole patch clamp experiments shown in Fig. 1B, chloroform strongly and reversibly activated TREK-1 expressed in transfected COS cells, while it slightly depressed TASK and did not effect TRAAK. As shown in Fig. 1C, chloroform induced a typical background current, characterized by an outward-going rectification which reverses at the predicted value for  $E_K^+$  as demonstrated by the shift of the reversal potential to 0 mV in symmetrical K<sup>+</sup> conditions. No current activation was observed in mock-transfected cells (Fig. 1B). Fig. 1D shows that chloroform also reversibly and reproducibly hyperpolarized COS cells expressing TREK-1 (but not mock-transfected

cells, not shown; n = 5). TREK-1 activation was dose dependent, with a threshold concentration for activation of 500  $\mu$ M, as shown in Fig. 1E. The inset of Fig. 1E shows that current activation, measured at a holding potential of 0 mV, started rapidly, but steady-state activation was barely reached after one minute in the presence of chloroform.

Both TREK and TASK, but not TRAAK, were opened by halothane. As shown in Fig. 2B and 2C, halothane-induced current displayed an outward-going rectification and reversed at the predicted value for  $E_K^+$ . The dose-effect curves (shown in Fig. 2D and Fig. 2E) demonstrated that the threshold concentrations for halothane on TREK-1 and TASK are 400  $\mu$ M and 200  $\mu$ M, respectively. The effects of halothane on TASK were rapid completely reversible. Opening of TASK was faster compared to the opening of TREK-1 by chloroform (compare Fig. 2F with Fig. 1E, inset). These results suggest that the components of the activation of both channels by anesthetics may be mediated by different molecular mechanisms.

Isoflurane, like halothane, activated both TREK-1 and TASK channels without altering TRAAK, as shown in Fig. 3A. Diethyl ether, like chloroform, opened TREK-1, but did not affect TRAAK. Diethyl ether also decreased TASK activity.

To demonstrate that activation by volatile anesthetics does not occur via second messenger pathways, experiments were conducted on excised patches. Fig. 4A shows that in an excised outside-out patch, halothane reversibly, and in a dose dependent manner, opened a 48 pS TREK-1 channel. No channel activity was observed in the absence of the anesthetic, suggesting that halothane converts inactive channels into active channels. The I-V curve of the chloroform-sensitive current in an outside-out patch shows the characteristic outward-going rectification previously observed in the macroscopic whole cell conditions, as shown in Figs. 4B-4C and Fig. 1B (inset). As

shown in Figs. 4D-4E, in the inside-out patch configuration, halothane reversibly opened a 12 pS TASK channel. In the absence of anesthetics, a single TASK channel was opened, as shown in the left panels of Figs. 4D-4E. The addition of halothane induced the opening of a second channel (see Figs. 4D-4E middle panel) which closed again after washing (see right panel of Figs. 4D and 4E).

5 All the data, taken together, demonstrate that volatile general anesthetics open TASK and TREK-1 channels and that these effects are likely to be direct and independent of second messengers.

TREK-1 and TASK are probably critical channels for the action of general volatile anesthetics. Opening of these K<sup>+</sup> channels along with the known modulation of neurotransmitter receptors will probably explain general anesthetic action. Effects of volatile anesthetics on ligand-gated ion channels such as GABA<sub>A</sub> receptors are probably particularly important at the post-synaptic level. Opening of background TREK-1 and TASK channels by volatile anesthetics might be very important both at the pre-synaptic ( $I_{K(An)}$ ) and at the post-synaptic level. An activation of only a small fraction of these  $I_{K(An)}$  channels will be expected to have significant effects on membrane polarization and consequently potentially important effects in both pre- and post-synaptic functions. TREK-1 and TASK channels are expressed in neuronal cells but they are also expressed in other tissues, and particularly in the heart. It is, therefore, not surprising that volatile anesthetics have depressive side-effects on heart function. These effects include a slowing of the heart rate and negative inotropic effects and are fully compatible with an exaggerate opening of background K<sup>+</sup> channels by volatile anesthetics. The same considerations might very well explain ventilatory depression.

What is claimed is:

1. An isolated nucleic acid molecule encoding the human TREK-1 potassium channel comprising the nucleic acid sequence of SEQ ID NO:1.
2. An isolated nucleic acid molecule encoding a human TREK-1 potassium channel, wherein said TREK-1 potassium channel comprises the amino acid sequence of SEQ ID NO:2.
3. An isolated human TREK-1 potassium channel comprising the amino acid sequence of SEQ ID NO:2.
4. The isolated human TREK-1 potassium channel of claim 3 comprising an amino acid sequence that is at least 99% identical to SEQ ID NO:2.
5. The isolated human TREK-1 potassium channel of claim 3 comprising an amino acid sequence that is at least 97% identical to SEQ ID NO:2.
6. The isolated human TREK-1 potassium channel of claim 3 comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:2.
7. An isolated nucleic acid molecule encoding the murine TREK-1 potassium channel comprising the nucleic acid sequence of SEQ ID NO:3.
8. An isolated nucleic acid molecule encoding a murine TREK-1 potassium channel, wherein said TREK-1 potassium channel comprises the amino acid sequence of SEQ ID NO:4.
9. The isolated murine TREK-1 potassium channel of claim 8 comprising the amino acid sequence of SEQ ID NO:4.
10. The isolated murine TREK-1 potassium channel of claim 8 comprising an amino acid sequence that is at least 99% identical to SEQ ID NO:4.

11. The isolated murine TREK-1 potassium channel of claim 8 comprising an amino acid sequence that is at least 97% identical to SEQ ID NO:4.
12. The isolated murine TREK-1 potassium channel of claim 8 comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:4.
13. A method for identifying substances having anesthetic properties, wherein said substances produce a safe, reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:
  - (a) contacting said substance with a mammalian potassium transport protein, wherein said potassium transport protein exhibits outward-going potassium rectification; and
  - (b) determining the potassium transport activity of said potassium transport protein, wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.
14. The method of claim 13, wherein said potassium transport protein is TASK.
15. The method of claim 13, wherein said potassium transport protein is TREK-1.
16. The method of claim 15, wherein said TREK-1 comprises the amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.
17. The method of claim 15, wherein said mammalian potassium transport protein is a chimeric molecule comprising at least a portion of murine TREK-1 and at least a portion of human TREK-1 wherein said chimeric molecule comprises 2P domains and at least 4 transmembrane domains.
18. A method for identifying substances having anesthetic properties, wherein said substances produce a safe, reversible state of unconsciousness with concurrent amnesia and analgesia

in a mammal upon inhalation comprising:

(a) contacting said substance with COS cells, wherein said COS cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding TREK-1, wherein said COS cells transiently express said TREK-1 on a surface of said COS cells, and wherein TREK-1 exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said TREK-1 wherein an activation of potassium transport is indicative of said substance having said anesthetic properties.

19. The method of claim 18, wherein said TREK-1 comprises an amino acid sequence that is at least 95% identical to SEQ ID NO:2.
20. The method of claim 18, wherein said TREK-1 comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 4.
21. The method of claim 18, wherein said TREK-1 comprises a chimeric molecule comprising at least a portion of human TREK-1 and at least a portion of murine TREK-1.
22. A method for identifying substances having anesthetic properties, wherein said substances produce a safe, reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation comprising:

(a) contacting said substance with transfected cells, wherein said transfected cells are transfected with a nucleotide vector comprising a nucleic acid molecule encoding TASK, wherein said transfected cells transiently express said TASK on a surface of said transfected cells, and wherein TASK exhibits outward-going potassium rectification; and

(b) determining the potassium transport activity of said TASK wherein an activation

of potassium transport is indicative of said substance having said anesthetic properties.

23. The method of claim 22, wherein said TASK comprises an amino acid sequence that is at least 95% identical to SEQ ID NO:5.
24. The method of claim 22, wherein said TASK is a chimeric molecule comprising at least a portion of TASK and at least a portion of another potassium transport protein selected from the group consisting of human TREK-1 and murine TREK-1, wherein said chimeric molecule comprises 2P domains and at least 4 transmembrane domains.
25. The method of claim 22, wherein said transfected cells are selected from the group consisting of COS cells, HELA cells, *Spodoptera* cells, *Xenopus* oocytes, embryonic kidney cells, Chinese hamster ovary cells, and fibroblasts.

## ABSTRACT OF THE DISCLOSURE

Human and mouse TREK-1 potassium transport proteins are disclosed as well a method for using potassium transport proteins for identifying substances having anesthetic properties, such as producing a safe, reversible state of unconsciousness with concurrent amnesia and analgesia in a mammal upon inhalation, in a method comprising (a) contacting the test substance with a mammalian potassium transport protein, wherein said potassium transport protein exhibits outward-going potassium rectification; and (b) determining the potassium transport activity of the potassium transport protein, wherein an activation of potassium transport is indicative of the test substance having anesthetic properties.

Figures 1A -1E

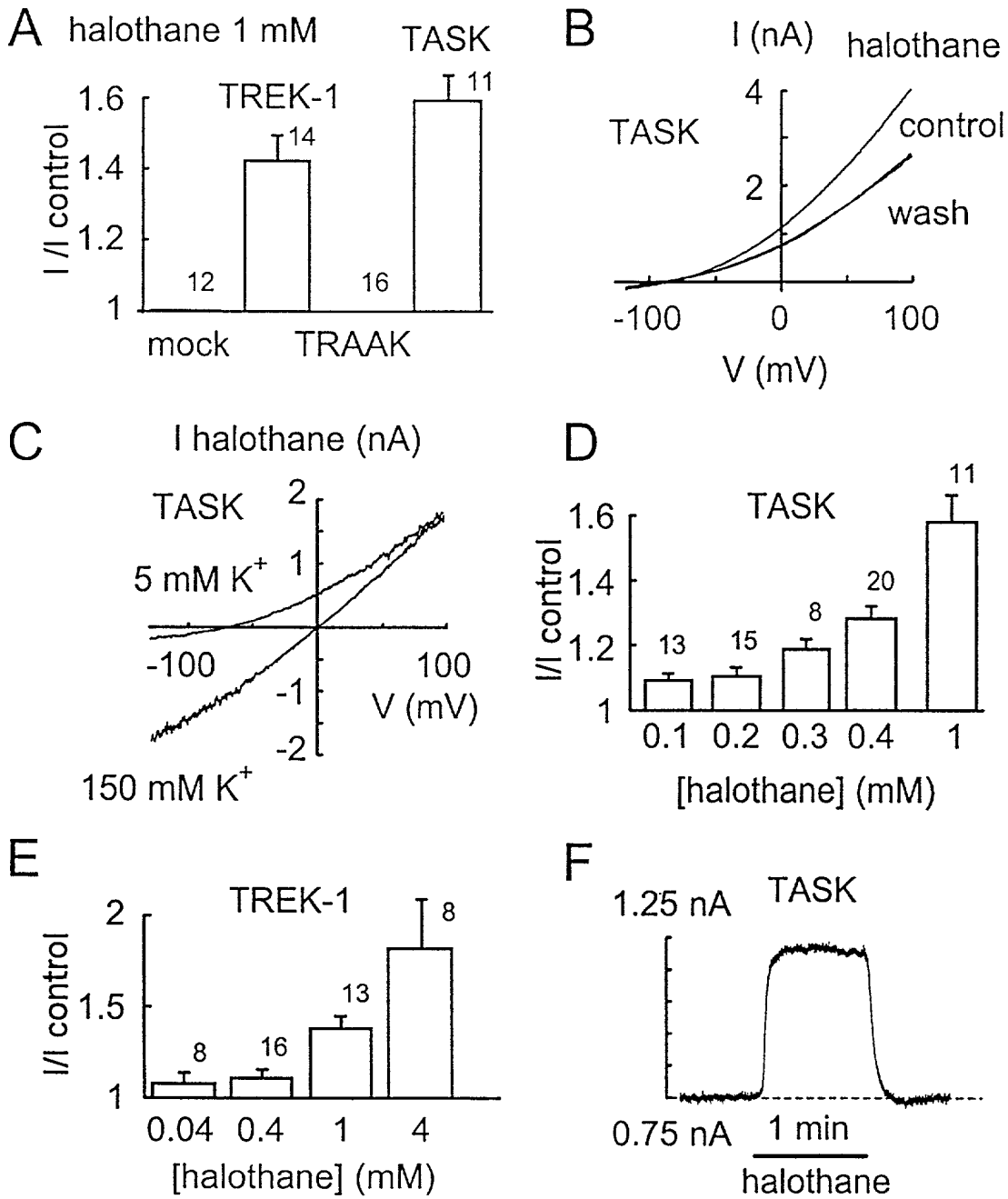


Figure 2A-2F

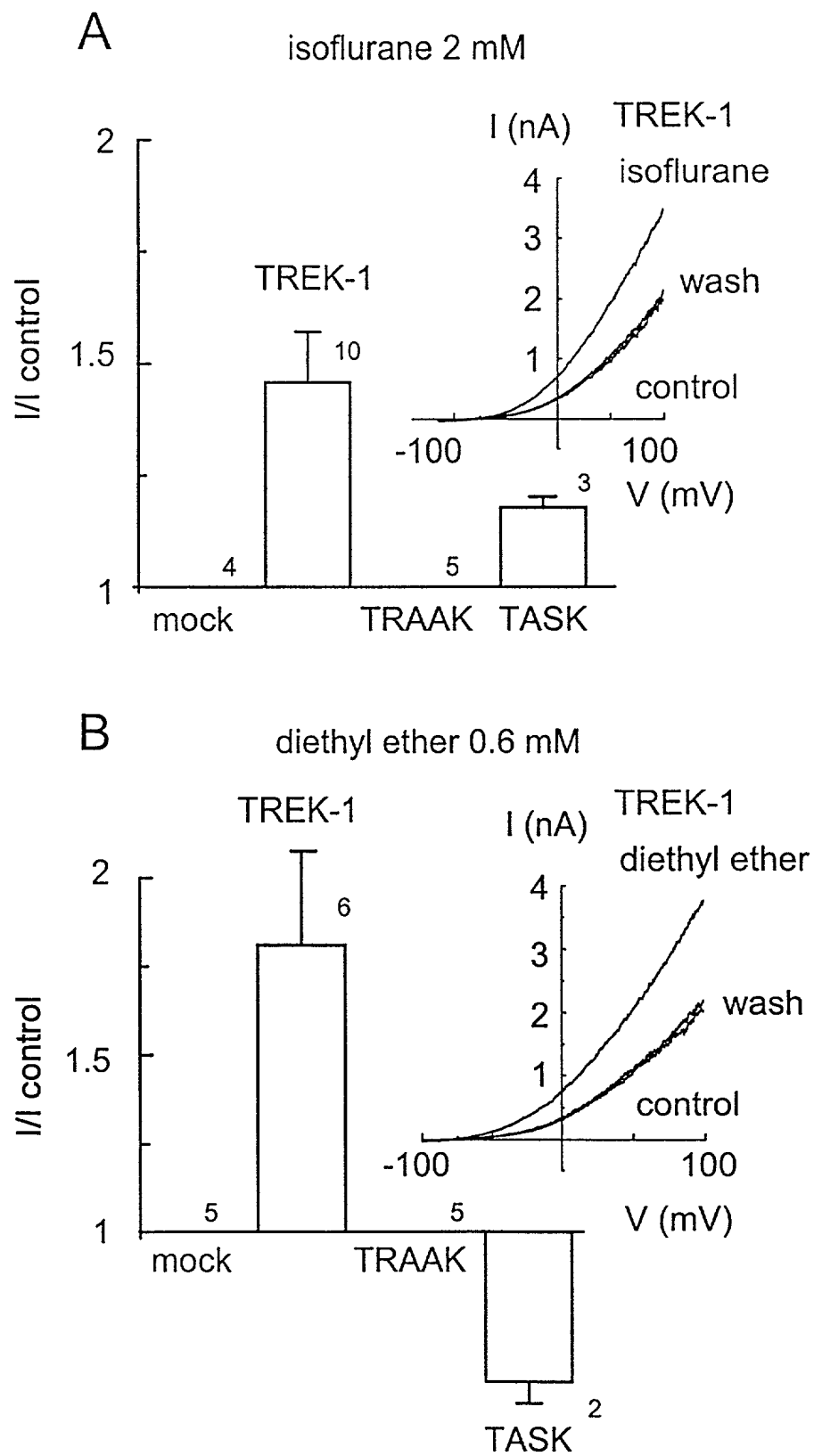


Figure 3A-B

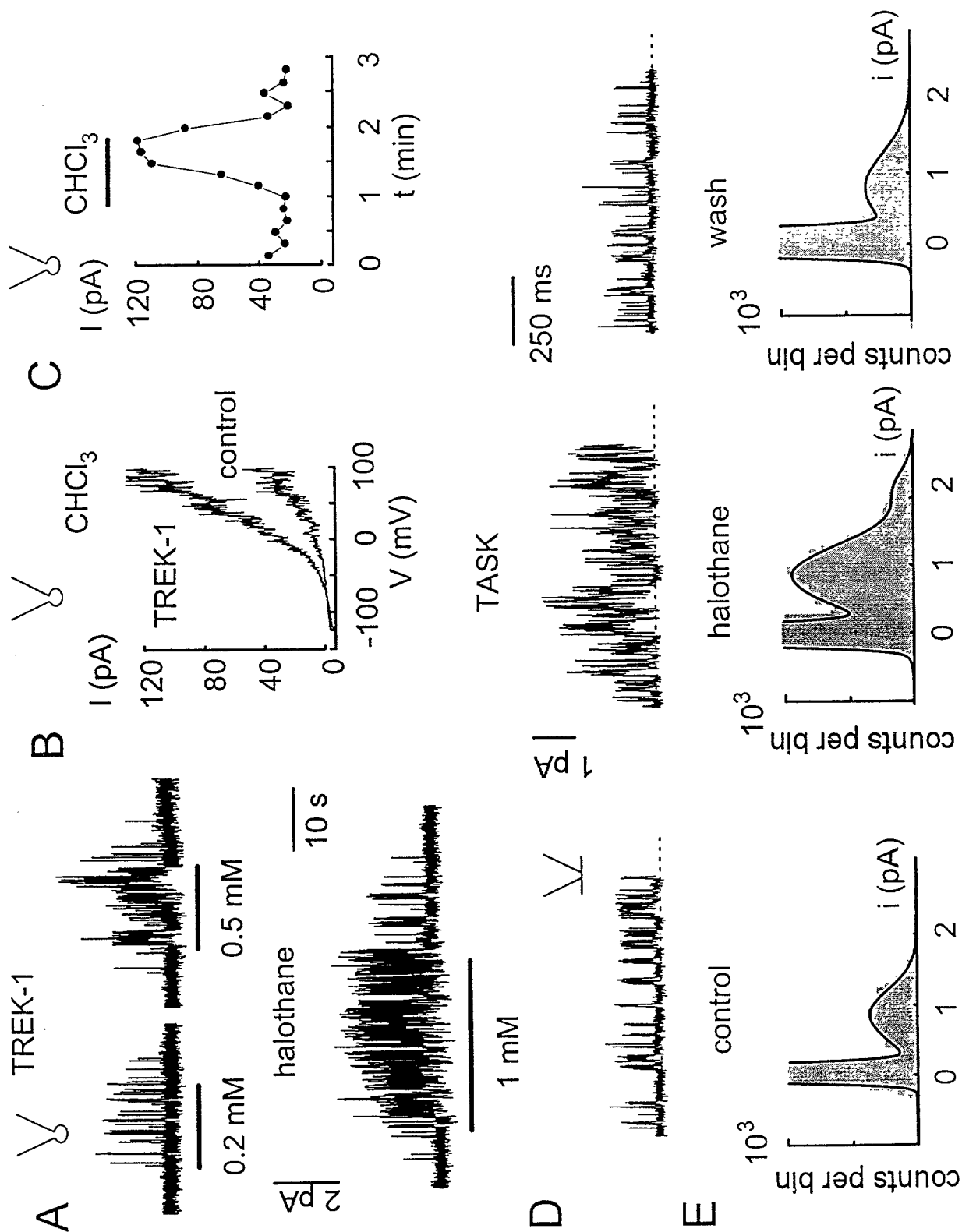


Figure 4A-4E

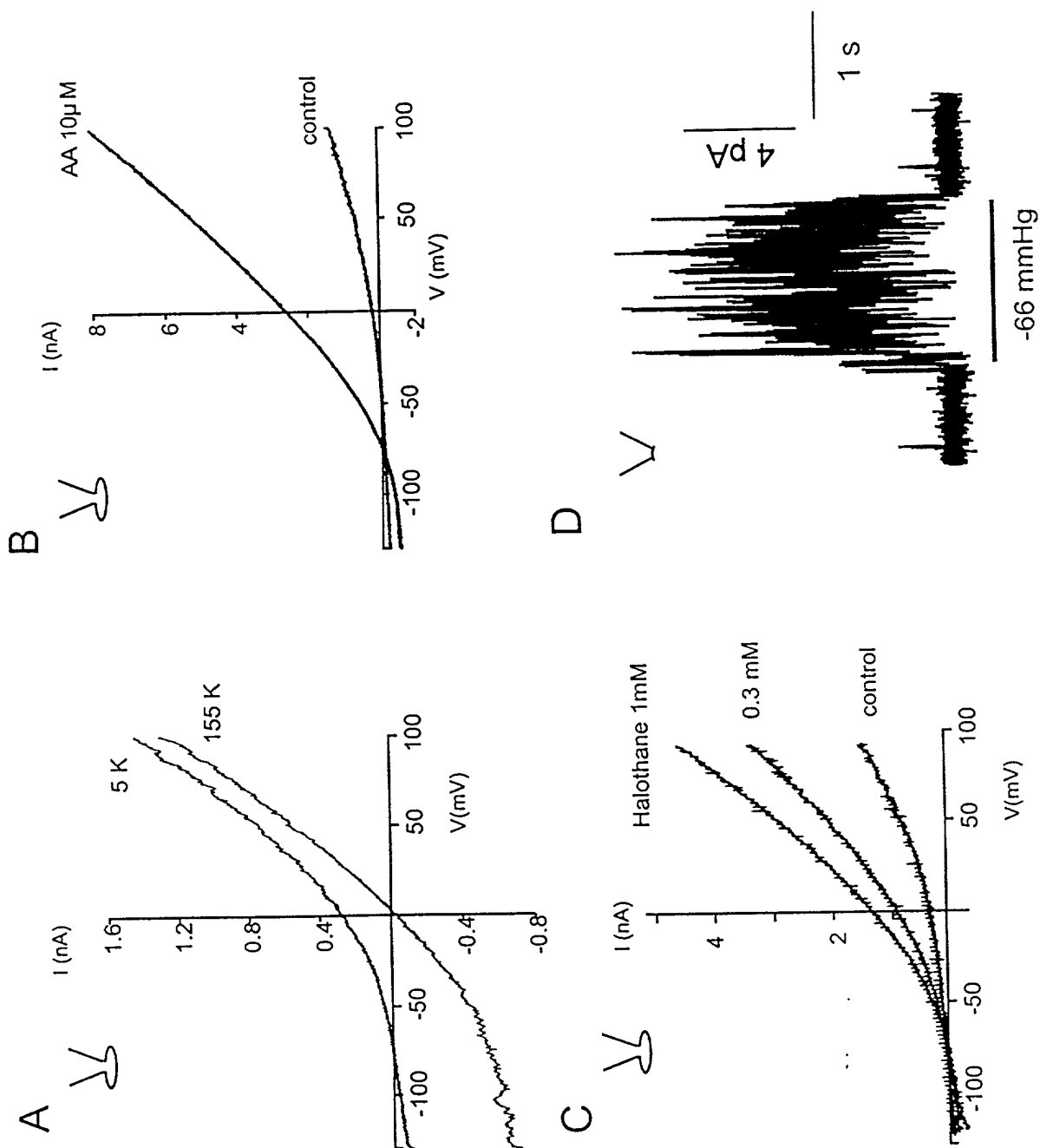


FIGURE 5A-D

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**DECLARATION FOR UTILITY OR  
DESIGN  
PATENT APPLICATION  
(37 CFR 1.63)**

☒ Declaration Submitted with Initial Filing      OR      ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

**Attorney Docket Number** 1030-R-00  
**First Named Inventor** Amanda J. PATEL

**COMPLETE IF KNOWN**

**Application Number** Not yet known  
**Filing Date** February 11, 2000  
**Group Art Unit** Not yet assigned  
**Examiner Name** Not yet assigned

As a below named inventor, I hereby declare

My residence, post office address, and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A METHOD FOR THE IDENTIFICATION OF ANESTHETICS

the specification of which (Title of the Invention)

☒ is attached hereto  
OR

☐ was filed on (MM/DD/YYYY)

as United States Application Number or PCT International

Application Number Not yet known and was amended on (MM/DD/YYYY) (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

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☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

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Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.
60/119,727	02/12/99	

[Page 1 of 2]

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
09/144,914	09/01/98	

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Country		Telephone		Fax	

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Inventor's Signature					Date		
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<input checked="" type="checkbox"/> Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto							

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Post Office Address							
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Inventor's Signature					Date			
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Given Name (first and middle [if any])				Family Name or Surname				
Inventor's Signature					Date			
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# SEQUENCE LISTING

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HONORE, ERIC  
LESAGE, FLORIAN  
ROMEY, GEORGES  
LAZDUSKI, MICHEL

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tcc aaa ccg agg ctc tca ttc tcc tca aaa ccc acc gtg ctt gct tcc 576  
Ser Lys Pro Arg Leu Ser Phe Ser Ser Lys Pro Thr Val Leu Ala Ser  
20 25 30  
cgg gtg gag agt gac tcg gcc att aat gtt atg aaa tgg aag aca gtc 624  
Arg Val Glu Ser Asp Ser Ala Ile Asn Val Met Lys Trp Lys Thr Val  
35 40 45  
tcc acg att ttc ctg gtg gtc gtc ctc tac ctg atc atc gga gcc gcg 672  
Ser Thr Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Ala  
50 55 60  
gtg ttc aag gca ttg gag cag cct cag gag att tcc cag agg acc acc 720  
Val Phe Lys Ala Leu Glu Gln Pro Gln Glu Ile Ser Gln Arg Thr Thr  
65 70 75  
att gtg atc cag aag cag acc ttc ata gcc cag cat gcc tgc gtc aac 768  
Ile Val Ile Gln Lys Gln Thr Phe Ile Ala Gln His Ala Cys Val Asn  
80 85 90 95  
tcc acc gag ctg gac gaa ctc atc cag caa ata gtg gca gca ata aac 816  
Ser Thr Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn  
100 105 110  
gca ggg att atc ccc tta gga aac agc tcc aat caa gtt agt cac tgg 864  
Ala Gly Ile Ile Pro Leu Gly Asn Ser Ser Asn Gln Val Ser His Trp  
115 120 125  
gac ctc gga agc tct ttc ttc ttt gct ggt act gtt atc aca acc ata 912  
Asp Leu Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile  
130 135 140  
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Gly Phe Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys	
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Ile Ile Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala	
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Gly Val Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys	
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gtg gaa gac aca ttt att aag tgg aat gtt agt cag acg aag att cgt	1104
Val Glu Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg	
195 200 205	
atc atc tcc acc atc atc ttc atc ctg ttt ggc tgt gtc ctc ttt gtg	1152
Ile Ile Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val	
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Ala Leu Pro Ala Val Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu	
225 230 235	
gac gct atc tat ttt gtg gtt atc act ctg acg acc att gga ttt gga	1248
Asp Ala Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly	
240 245 250 255	
gac tac gtg gca ggt gga tca gac att gaa tat ctg gac ttc tac aag	1296
Asp Tyr Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys	
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Pro Val Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala	
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gtt ctg agc atg att ggg gac tgg cta cgg gtg atc tct aag aag acg	1392
Val Leu Ser Met Ile Gly Asp Trp Leu Arg Val Ile Ser Lys Lys Thr	
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Lys Glu Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala	
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aat gtc acg gcc gag ttc aag gaa acg agg agg cgg ctg agc gtg gag	1488
Asn Val Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu	
320 325 330 335	
atc tac gac aag ttc cag cgt gcc aca tcc gtg aag cgg aag ctc tcc	1536
Ile Tyr Asp Lys Phe Gln Arg Ala Thr Ser Val Lys Arg Lys Leu Ser	
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Ala Glu Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg	
355 360 365	
acc ctg tct gtg aac cac ctg acc agc gag agg gaa gtc ctg cct ccc	1632
Thr Leu Ser Val Asn His Leu Thr Ser Glu Arg Glu Val Leu Pro Pro	
370 375 380	
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Leu Leu Lys Ala Glu Ser Ile Tyr Leu Asn Gly Leu Thr Pro His Cys	
385 390 395	
gct ggt gag gac ata gct gtc att gag aac atg aag tag ccctctcttg	1729

Ala Gly Glu Asp Ile Ala Val Ile Glu Asn Met Lys  
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Val Glu Ser Asp Ser Ala Ile Asn Val Met Lys Trp Lys Thr Val Ser

35 40 45

Thr Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Ala Val

50 55 60

Phe Lys Ala Leu Glu Gln Pro Gln Glu Ile Ser Gln Arg Thr Thr Ile

65 70 75 80

Val Ile Gln Lys Gln Thr Phe Ile Ala Gln His Ala Cys Val Asn Ser

85 90 95

Thr Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala

100 105 110

Gly Ile Ile Pro Leu Gly Asn Ser Ser Asn Gln Val Ser His Trp Asp

115 120 125

Leu Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly

130 135 140

Phe Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile

145 150 155 160

Ile Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly

165 170 175

Val Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val

180 185 190

Glu Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile

195 200 205

Ile Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala

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Leu Pro Ala Val Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp			
225	230	235	240
Ala Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp			
	245	250	255
Tyr Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro			
	260	265	270
Val Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val			
	275	280	285
Leu Ser Met Ile Gly Asp Trp Leu Arg Val Ile Ser Lys Lys Thr Lys			
	290	295	300
Glu Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn			
305	310	315	320
Val Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile			
	325	330	335
Tyr Asp Lys Phe Gln Arg Ala Thr Ser Val Lys Arg Lys Leu Ser Ala			
	340	345	350
Glu Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr			
	355	360	365
Leu Ser Val Asn His Leu Thr Ser Glu Arg Glu Val Leu Pro Pro Leu			
	370	375	380
Leu Lys Ala Glu Ser Ile Tyr Leu Asn Gly Leu Thr Pro His Cys Ala			
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20 25 30  
Pro Glu Leu Ile Glu Arg Gln Arg Leu Glu Leu Arg Gln Gln Glu Leu  
35 40 45  
Arg Ala Arg Tyr Asn Leu Ser Gln Gly Gly Tyr Glu Glu Leu Glu Arg  
50 55 60  
Val Val Leu Arg Leu Lys Pro His Lys Ala Gly Val Gln Trp Arg Phe  
65 70 75 80  
Ala Gly Ser Phe Tyr Phe Ala Ile Thr Val Ile Thr Thr Ile Gly Tyr  
85 90 95  
Gly His Ala Ala Pro Ser Thr Asp Gly Gly Lys Val Phe Cys Met Phe  
100 105 110  
Tyr Ala Leu Leu Gly Ile Pro Leu Thr Leu Val Met Phe Gln Ser Leu  
115 120 125  
Gly Glu Arg Ile Asn Thr Leu Val Arg Tyr Leu Leu His Arg Ala Lys  
130 135 140  
Lys Gly Leu Gly Met Arg Arg Ala Asp Val Ser Met Ala Asn Met Val  
145 150 155 160  
Leu Ile Gly Phe Phe Ser Cys Ile Ser Thr Leu Cys Ile Gly Ala Ala  
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Ala Phe Ser His Tyr Glu His Trp Thr Phe Phe Gln Ala Tyr Tyr Tyr  
180 185 190  
Cys Phe Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp Tyr Val Ala Leu  
195 200 205  
Gln Lys Asp Gln Ala Leu Gln Thr Gln Pro Gln Tyr Val Ala Phe Ser  
210 215 220  
Phe Val Tyr Ile Leu Thr Gly Leu Thr Val Ile Gly Ala Phe Leu Asn  
225 230 235 240  
Leu Val Val Leu Arg Phe Met Thr Met Asn Ala Glu Asp Glu Lys Arg  
245 250 255

